the substrate, which is turn is dependent on the size of the area of contact between the cell membrane and the substrate.

The physical area of contact between the cell membrane and a planar silicon chip (about 1μm width of contact rim; see Figure 2, right hand diagram) with a smoothly rounded, funnel-like orifice is much larger than that formed between a cell membrane and a glass micropipette (about 100 nm width; Figure 2, left hand diagram). This results in the force per unit area being considerably reduced in the chip relative to the pipette configuration, and the number of intercalated glycoproteins in the contact area being much larger, effectively preventing the required Angström distance between the phospholipid bilayer and the substrate surface imperative for the formation of a gigaseal.

The present invention seeks to address this problem by providing a planar substrate (e.g. a silicon-based chip), suitable for patch clamp studies of the electrophysiological properties of cell membrane, which is designed to provide a reduced area of contact with the cell membrane, thereby promoting the formation of a gigaseal.

Thus, a first aspect of the invention provides a substantially planar substrate for use in patch clamp analysis of the electrophysiological properties of a cell membrane comprising a glycocalyx, wherein the substrate comprises an aperture having a rim defining the aperture, the rim being adapted to form a gigaseal upon contact with the cell membrane.

In a preferred embodiment, the substrate is a silicon-based chip.

In the present context, the term gigaseal normally indicates a seal of a least 1G ohm, and this is the size of seal normally aimed at as a minimum, but for certain types of measurements where the currents are large, lower values may be sufficient as threshold values.

By 'glycocalyx' we mean the layer created by the peptide- and glyco-moieties, which extend into the extracellular space from the glycoproteins in the lipid bilayer of the cell membrane.

Preferably, the rim protrudes from the plane of the substrate to a length in excess of the height of the glycocalyx above the phospholipid bilayer of the cell membrane. More preferably, the rim extends at least 20nm, at least 30 nm, at least 40 nm, at least 50 nm, at least 60 nm, at least 70 nm, at least 80 nm, at least 90 nm or at least 100 nm above the plane of the substrate

Advantageously, the rim is shaped such that the area of physical contact between the substrate and the cell membrane is minimised, thereby favouring penetration of the glycocalyx and formation of a gigaseal.

It will be appreciated by persons skilled in the art that the rim may be of any suitable cross-sectional profile. For example, the walls of the rim may be tapered or substantially parallel . Likewise, the uppermost tip of the rim may take several shapes, for example it may be dome-shaped, flat or pointed. Furthermore, the rim protrusion may be substantially perpendicular to, oblique, or parallel with the plane of the substrate. A parallel protruding rim may be located at or near to the mouth of the aperture or, alternatively, positioned deeper into the aperture. Conveniently, the width of the rim is between 10 and 200 nm.

Alternatively the rim is formed by the mouth of the aperture itself, rather from a protrusion. The mouth of the aperture may be sharp with a radius of curvature between 5 and 100 nm with an angle of 45 to 90 degrees.

It will be further appreciated by persons skilled in the art that the aperture should have dimensions which do not permit an intact cell to pass through the planar substrate.

Examples of the general design of the preferred embodiment of the first aspect of the invention wherein the substrate comprises integral electrodes (but without the rimmed aperture feature of the present invention) are described in WO 01/25769.

A second aspect of the invention provides a method for making a substrate according to the first aspect of the invention, the method comprising the steps of

(i) providing a substrate template;

(ii) forming an aperture in the template; and

(iii) forming a rim around the aperture.

Preferably, the substrate is manufactured using silicon micro fabrication technology "Madou, M., 2001".

It will be appreciated by persons skilled in the art that steps (ii) and (iii) may be performed sequentially (i.e. in temporally separate steps) or at the same time.

Advantageously, step (ii) comprises forming an aperture by use of an inductively coupled plasma (ICP) deep reactive ion etch process. "Laermer F. and Schilp, A., DE4241045"

When it is required to form a substantially vertical protrusion relative to the plane of the substrate, the method comprises an intermediate step of a directional and selective etching of the front side of the substrate causing a removal of a masking layer on the front side of the substrate, and further proceeding the prescribed protrusion distance into the underlying substrate.

As a result of a faster etch rate of silicon compared to that of the masking material, the masking material will be left inside the aperture, and protrude from the surface. An overall surface coating can subsequently be applied.

When it is required to form a protrusion lying substantially in the plane of the substrate, the method comprises an intermediate step of using Inductively Coupled Plasma (ICP) etch or Advanced Silicon Etch (ASE) for the formation of the pore, where the repetitive alternation of etching and passivation steps characterising these methods, will result in some scalloping towards the mouth of the aperture. By suitable adjustment of the process parameters, the scalloping can result in an inward in plane protrusion of the rim.

Again, an overall surface coating can subsequently be employed.

Conveniently, the method further comprises coating the surface of the substrate (e.g. with silicon oxide), either before or after formation of the aperture and/or rim. Alternatively, step (iii) is performed at the same time as coating the substrate.

Such coatings may be deposited by use of plasma enhanced chemical vapour deposition (PECVD) and/or by use of low pressure chemical vapour deposition (LPCVD).

The preferred embodiment of the first aspect of the invention wherein the substrate comprises integral electrodes may be manufactured as described in WO 01/25769).

A third aspect of the invention provides a method for analysing the electrophysiological properties of a cell membrane comprising a glycocalyx, the method comprising

(i) providing a substrate having a rimmed aperture according to the first aspect of the invention;

(ii) contacting the cell membrane with the rim of an aperture of the substrate such that a gigaseal is formed between the cell membrane and the substrate; and

(iii) measuring the electrophysiological properties of the cell membrane.

In a preferred embodiment of the third aspect of the invention, there is provided a method of establishing a whole cell measuring configuration for determining and/or monitoring an electrophysiological property of one or more ion channels of one or more ion channel-containing structures, said method comprising the steps of:

(i) providing a substrate as defined above;

(ii) supplying a carrier liquid at one or more apertures, said carrier liquid containing one or more ion channel-containing structures;

(iii) positioning at least one of the ion channel-containing structures at a corresponding number of apertures;

(iv) checking for a high electrical resistance seal between an ion channel-containing structure held at a site (i.e. aperture) and the surface part of the site (i.e. rim) with which the high electrical resistance seal is to be provided by successively applying a first electric potential difference between the measuring electrode associated with the site and a reference electrode, monitoring a first current flowing between said measuring electrode and said reference electrode, and comparing said

provided. The person skilled in the art will be able to select such suitable measuring circuit.

A fourth aspect of the invention provides a kit for performing a method according to the third aspect of the invention, the kit comprising a substrate according to the first aspect of the invention and one or more media or reagents for performing patch clamp studies.

Preferably the kit comprises a plurality of substrates.

The invention will now be described with reference to the following non-limiting examples and figures:

Figure 1 shows the cell with a patch pipette attached. In the gigaseal zone, (indicated by shaded area at point of contact between the pipette tip and the cell membrane) the glycoproteins of the glycocalyx have been displaced laterally to allow direct contact between the membrane phospholipid bilayer and the pipette;

Figures 2a and 2b show a cell attached to either a pipette tip (Figure 2a) or a planar substrate (Figure 2b). The area of contact between the cell membrane and substrate surface is considerably larger in the substrate configuration (Figure 2b) than in the pipette configuration (Figure 2a).

Figure 3 shows the variation in actual pipette resistance for each intended resistance set;

Figure 4 shows Gigaseal success rate versus pipette resistance;

Figure 5 shows the success rate of whole-cell establishment (from successful gigaseals) versus pipette resistance;

Figure 6 shows the time-dependence of gigaseal formation with different aperture sizes, the error bars indicating the standard deviation from the mean;

CLAIMS

1. A substantially planar substrate for use in patch clamp analysis of the electrophysiological properties of a cell membrane comprising a glycocalyx, wherein the substrate comprises an aperture having a rim defining the aperture, the rim being adapted to form a gigaseal upon contact with the cell membrane.

2. A planar substrate according to Claim 1 wherein the rim protrudes from the plane of the substrate to a height in excess of the thickness of the glycocalyx.

3. A planar substrate according to Claim 1 or 2 wherein the rim protrudes from the plane of the substrate to a height of at least 20 nm above the surface of the planar substrate, preferably least 30 nm, at least 40 nm, at least 50 nm, at least 60 nm, at least 70 nm, at least 80 nm, at least 90 nm or at least 100 nm.

4. A planar substrate according to any one of the preceding claims wherein the width of the rim is in the range 50 to 200 nm.

5. A planar substrate according to any of the preceding claims, in which the length (i.e. depth) of the aperture is between 2 and 30 μm, preferably between 2 and 20 μm, 2 and 10 μm, or 5 and 10 μm.

6. A planar substrate according to any of the preceding claims wherein the diameter of the aperture is in the range 0.5 to 2 μm.

7. A planar substrate according to any one of the preceding claims wherein the rim extends substantially perpendicularly to the plane of the substrate.

8. A substrate according to any one of Claims 1 to 6 wherein the rim forms an oblique angle with the plane of the substrate.

9. A substrate according to any one of Claims 1 to 6 wherein the rim is substantially parallel to the plane of the substrate.

10. A substrate according to Claim 1 wherein the rim is defined by a mouth of the aperture, which mouth has a radius of curvature between 5 and 100nm with an angle of 45 to 90 degrees.

11. A planar substrate according to any of the preceding claims wherein the substrate is made of silicon, plastics, pure silica or other glasses, such as quartz and PyrexTM, or silica doped with one or more dopants selected from the group of Be, Mg, Ca, B, Al, Ga, Ge, N, P, As.

12. A planar substrate according to Claim 11 wherein the substrate is made of silicon.

13. A substrate according to any of the preceding claims wherein the surface of the substrate and/or the walls of the aperture are coated with a second coating material.

14. A substrate according to Claim 13 wherein the coating material is silicon, plastics, pure silica, other glasses such as quartz and PyrexTM, silica doped with one or more dopants selected from the group of Be, Mg,

15. A substrate according to Claim 11 wherein the coating material is silicon oxide.

16. A method of making a substrate according to any one of Claims 1 to 15 comprising, the method comprising the steps of

(i) providing a substrate template;

(ii) forming an aperture in the template; and

(iii) forming a rim around the aperture.

17. A method according to Claim 16 wherein the substrate is manufactured using silicon micro fabrication technology.

18. A method according to Claim 16 or 17 wherein step (ii) comprises forming an aperture by use of an inductively coupled plasma (ICP) deep reactive ion etch process.

19. A method according to any one of Claims 16 to 18 further comprising the step of coating the surface of the substrate.

20. A method according to Claim 19 wherein step (iii) is performed at the same time as coating the substrate.

21. A method according to Claim 19 wherein step (iii) comprises an intermediate step of a directional and selective etching of the front side of the substrate causing a removal of a masking layer on the front side of the

substrate, and further proceeding the prescribed protrusion distance into the underlying substrate.

22. A method according to Claim 19, 20 or 21 wherein the coating is deposited by use of plasma enhanced chemical vapour deposition (PECVD) and/or by use of low pressure chemical vapour deposition (LPCVD).

23. A method according to Claim 22 wherein the coating is deposited by use of plasma enhanced chemical vapour deposition (PECVD).

24. A method according to Claim 18 wherein step (iii) comprises forming a rim from a deposited surface coating by use of plasma enhanced chemical vapour deposition (PECVD).

25. A method for analysing the electrophysiological properties of a cell membrane comprising a glycocalyx, the method comprising the following steps:

(i) providing a substrate having a rimmed aperture according to any one of Claims 1 to 15;

(ii) contacting the cell membrane with the rim of an aperture of the substrate such that a gigaseal is formed between the cell membrane and the substrate; and

(iii) measuring the electrophysiological properties of the cell membrane.

26. A kit for performing a method according to Claim 25, the kit comprising a substrate according to any one of Claims 1 to 15 and one or

27. A substrate substantially as herein before described with reference to the accompanying drawings.

28. A method substantially as herein before described with reference to the accompanying drawings.

29. A kit substantially as herein before described with reference to the accompanying drawings.